Postharvest Biology and Technology 100 (2015) 52–58

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Effect of preharvest application of *Hanseniaspora uvarum* on postharvest diseases in strawberries

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ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form
24 September 2014
Accepted 27 September 2014
Available online 8 October 2014

Keywords:
Hanseniaspora uvarum
Strawberry
Defense-related enzymes

ABSTRACT

This study was conducted to evaluate the efficacy of preharvest applications of *Hanseniaspora uvarum* against postharvest mold decay of strawberry (*Fragaria ananassa*) fruit and quality maintenance during storage at $2 \pm 1\,^{\circ}C$ and 90–95% RH. Results showed that the treatment significantly reduced postharvest mold decay ($P < 0.05$), maintained fruit firmness and total soluble solids content, and did not impair pH and surface color during postharvest storage. Morever, it was found that the activities of some defense-related enzymes, peroxidase, superoxide dismutase, catalase, polyphenoloxidase, phenylalanine ammonia-lyase, β-1,3-glucanase and ascorbate peroxidase, increased and malondialdehyde content, a decomposition product of polyunsaturated fatty acid hydroperoxides, was reduced, in response to preharvest application of *H. uvarum*. Overall, preharvest application of *H. uvarum* has potential for controlling postharvest decay of strawberry.

1. Introduction

In recent years, the use of biocontrol agents has become a new way for controlling postharvest diseases of fruit and vegetables. Among them, antagonistic yeasts are attracting research focus, with their wide antimicrobial spectrum, good antagonitic effects, genetic stability, low nutrition requirements and high security (Fan et al., 2009a; Zong et al., 2010; Mekbib et al., 2011; Yu et al., 2012). Yeasts that are naturally present on. fruit surfaces represent the major group and they have also been isolated from other sources, such as the phyllosphere, roots, soil, and sea water (Liu et al., 2013). The mechanisms of action of most biocontrol agents of postharvest diseases are poorly understood and it is generally assumed that they involve a complex interaction between host, pathogen, antagonists and environment (Nunes, 2012). The basic mechanism of action for most of antagonistic yeasts is competition for available nutrients and space (Liu et al., 2013), which often occurs in the first 24 h after the yeast cells come into contact with fruit surface. Other modes of action of yeast antagonists against specific fungal pathogens are: induction of host defense (Zheng and Chen, 2009; Xu et al., 2013), attachment and lytic enzyme secretion (Chan and Tian, 2005), adjustment of population density (McGuire, 2000; Fiori et al., 2012), morphology change (Fiori et al., 2012), reactive oxygen species (ROS) tolerance (Liu et al., 2011a), iron depletion (Saravanakumar et al., 2008), alleviation of oxidative damage of the fruit host (Xu et al., 2008), and induction of ROS production in the host (Macarisin et al., 2010).

Strawberry (*Fragaria* × *ananassa Duch.*) is a non-climacteric fruit with a very short postharvest life. Loss of quality in this fruit is mostly due to its relatively high metabolic activity and sensitivity to fungal decay, and the decay is mainly caused by gray mold (*Botrytis cinerea* Pers.) and rhizopus rot (*Rhizopus stolonifer* (Ehrenb.) Vuill.) (Romanazzi et al., 2001). Strawberry is also susceptible to water loss, bruising and mechanical injuries due to their soft texture and lack of a protective rind (Hernández-Muñoz et al., 2006). Several antagonistic yeasts against fruit rot in strawberry have been studied. El-Neshawy and Shetaia (2003) found the yeast *Candida oleophila* followed by *Candida fructus* provided significant decay control, with the greatest effect from *C. fructus* in restricting visual rating of mold development on strawberry and maintaining quality parameters, including firmness, soluble solid content, anthocyanin content, titratable acidity (TA), pH and surface color. Fan et al. (2009b) found a novel edible bio-film containing *Cryptococcus laurentii* significantly reduced microbial decay, decreased weight loss and maintained the firmness of strawberries. Karabulut et al. (2004) tested the yeast *Metschnikowia fructicola* for control of preharvest and postharvest rots of strawberry fruit and found it reduced the incidence of fruit rot significantly. Long and Yuan (2009) found preharvest treatment with the yeast *Kloeckera apiculata strain (34-9)* was the most effective to control *B. cinerea* on strawberry, while

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http://dx.doi.org/10.1016/j.postharvbio.2014.09.004
0925-5214/

postharvest and sumilex treatment equally reduced the incidence of decay. Zhang et al. (2007a) sprayed strawberry fruit with the antagonist *Rhodotorula glutinis* and found the higher the concentration of the antagonist, the lower the disease incidence. Zhang et al. (2007b) also found that the yeast *C. laurentii* was effective in controlling Rhizopus rot of strawberries and if combined with short hot water dips, the effect would be greater.

Although most postharvest diseases appear in the packinghouse, infections often begin in the field (Palou et al., 2002). Preharvest application can enhance the biocontrol system, because it will allow the antagonist to have longer interaction with the pathogen and to colonize tissues before the arrival of the pathogen, such as happens in latent and incipient infections occurring through wounds resulting from the harvesting period (Nunes, 2012). Therefore, spraying antagonistic yeasts before harvest to suppress fruit and vegetables postharvest storage diseases will be one of the important areas of the application of antagonistic microorganisms. *Hanseniaspora uvarum* was an effective antagonist against gray mold of grape and had inhibitory effect on spore germination and lesion growth of *B. cinerea* and reduced the natural decay development of grape berries, and did not impair quality parameters (Liu et al., 2010b). The objective of the present work was to assess the efficacy of preharvest application of the antagonistic yeast *H. uvarum* in inducing resistance against postharvest diseases and its effects on quality parameters of strawberries during storage.

2. Materials and methods

2.1. Preparation of suspensions of antagonist

H. uvarum (CGMCC 2.3970), isolated from strawberry fruit and identified based on a similarity analysis of its physiological–biochemical characteristics and 26S rDNA D1/D2 domain sequence, was cultured in 250 mL Erlenmeyer flasks with 100 mL of PDB (1 L distilled water containing potato 200 g and glucose 20 g) on a gyratory shaker at 180 rpm for 24 h at 28 °C. The cells were harvested by centrifuging at 10,000 rpm for 15 min at 4 °C, then washed with sterile-distilled water two times and resuspended in sterile distilled water. Cells concentration was counted with a haemocytometer and diluted to 1×10^8 CFU mL^{-1} with sterile-distilled water containing 0.05% Tween-20 as required.

2.2. Preharvest treatment of strawberry

Strawberries, cv. HongYan, were grown in a greenhouse located in Yuhua district, Nanjing city, Jiangsu province, China. Diurnal temperature in the greenhouses ranges from 8 °C to 25 °C. At 3 days before harvest in December, the fruit were sprayed with distilled water (as the control) and the yeast cell suspensions (1×10^8 CFU mL^{-1}) using a 500 mL watering can, until all fruit were wet to run-off. The watering can was soaked with 75% alcohol for 10 min and dried out an a clean bench before using. To ensure sufficient number of fruit for each treatment, no less than 400 were given different treatments and were separated by non-treated fruit. Commercially mature fruit were harvested after three days. Fruit free of wounds and homogeneous in maturity and size were selected, and then placed in polyethylene-lined plastic boxes and transported to storage room at 2 ± 1 °C and 90–95% RH. Each treatment contained three replications with 90 single berries per replication.

2.3. Effect of antagonist H. uvarum on decay index of strawberries

Fruit decay was determined on 40 fruit per sample according to a five-point scale, where 0 = no decay, 1 = very slight decay, covering <10% of the fruit surface, 2 = slight decay, covering >10% but <25% of the fruit surface, 3 = moderate decay, covering >25% but <40% of the fruit surface, and 4 = severe decay, covering >40% of the fruit surface. The decay index was calculated using the following formula:

$$\text{Decay index} = \frac{(1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4)}{4 \times N}$$

where N was the total number of fruit measured and N_1, N_2, N_3 and N_4 were the numbers of fruit showing the different severities of decay.

2.4. Effect of antagonist H. uvarum on fruit quality

Quality parameters were measured after storage on three replicates of ten fruit each. The testing methods are described below:

Fruit firmness was measured at two points on the equatorial region by using a TA-XT2i texture analyzer (Stable Micro Systems Ltd., UK) with a P_{50} cylinder plunger probe and the maximum force was recorded as fruit firmness (N). Test conditions used for measurements were: pre-test speed of 5.0 mm s^{-1}, test speed of 1.0 mm s^{-1}, and post-test speed of 5.0 mm s^{-1}, penetration distance of 4 mm.

Total soluble solids (TSS) were determined by measuring the refractive index of the strawberry fruit juice with a hand-held refractometer (WYT-4, Top instrument Co., Ltd., China) and the results expressed as percentages (g per 100 g fruit weight).

Surface color was measured at two points around the equatorial zone of the fruit by a Minolta CR-400 Chronometer (Konica Minolta Sensing, Osaka, Japan), using the CIE (Commission International de l'Eclairage) color space L^*, a^* and b^* values. Values of lightness L^* (ranging from 0, black to 100 white) and a^* (positives values for red, negative values for green) were measured.

pH was measured by using a pH meter (DELTA 320, Top instrument Co., Ltd., China) and strawberry juice filtered through one layer of cheesecloth was prepared for the measurement.

2.5. Effect of antagonist H. uvarum on MDA and defense-related enzyme activities of strawberry

At various time intervals (0, 3, 6, 9,12 and 15 days) after harvest, fruit peels were removed from five fruit in each treatment using a sterile scalpel to cut off peel of about 2 mm thickness, the achenes were discarded, and the skinless pulp tissues were frozen until assayed.

2.5.1. MDA content

Lipid peroxidation was determined in terms of malondialdehyde (MDA) content by the TBA reaction as described by Du and Bramlage (1992). Tissues (1 g) prepared were ground with 7 mL (V_1) 5% (w/v) cold trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. The solution contained 2 mL (V_2) of the resulting supernatant and 2 mL 0.67% (w/v) TBA was heated at 100 °C for 5 min, and quickly cooled in an ice-bath. The absorbance of the supernatants was recorded at A_{532} and A_{600}. MDA content was calculated according to the following formula: $C_{MDA}(\text{nmol g}^{-1}) = (A_{532} - A_{600}) \times V_1 / 1.55 \times 10^{-1} \times V_2 \times m$, where 1.55×10^{-1} was the molar extinction coefficient of MDA and m (g) was the weight of tissues prepared for measured.

2.5.2. SOD, POD and APX activity

For the determination of peroxidase (POD), superoxide dismutase (SOD) activity and ascorbate peroxidase (APX), skinless pulp tissues (2 g) prepared were ground with in 8 mL cold 50 mmol L^{-1} phosphate buffered saline solution (pH 7.8) containing 1% (w/v) polyvinyl–polypyrrolidone (PVPP). The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C, and 1 mL resulting supernatants were used as crude enzyme extracts for assaying

the enzyme activities. 1 mL guaiacol and 1 mL H_2O_2 were used as the substrates for POD activity determination (Kochba et al., 1977). Enzyme activity was defined as the increase in absorbance at 460 nm at 25 °C within 2 min. SOD activity was evaluated following the method of Luna (Luna et al., 1994). The reaction mixture was illuminated with a fluorescent lamp at 400 Lux for 10 min and the absorbance was measured at 560 nm. The same solutions held in the dark were used as the blank. One unit of SOD activity was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. APX activity was evaluated according to the method described by Nakano and Asada (1981). One unit was defined as the change in 0.001 absorbance units per minute at 290 nm. The specific activity was expressed as units per gram of fresh weight.

2.5.3. CAT activity

For the determination of catalase (CAT) activity, skinless pulp tissues (2 g) prepared were mixed with 8 mL of ice-cold 100 mmol L^{-1} sodium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinyl–polypyrrolidone (PVPP) and ground thoroughly. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. CAT activity determination was performed following the method of Wang et al. (2004). The reaction mixture contained 2 mL of sodium phosphate buffer, 0.4 mL of H_2O_2 and 1 mL of crude extract. The decomposition of H_2O_2 was measured at 240 nm with a spectrophotometer. One unit was defined as the change in 0.001 absorbance units per minute and the specific activity was expressed as units per gram of fresh weight.

2.5.4. PAL activity

For the determination of PAL activity, skinless pulp tissues (2 g) prepared were mixed with 8 mL of ice-cold 100 mmol L^{-1} boracic acid buffer (pH 8.7) containing 1% (w/v) polyvinyl–polypyrrolidone (PVPP), ground thoroughly and centrifuged at 10,000 rpm for 15 min at 4 °C. PAL activity was analyzed using the method of Assis et al. (2001). One unit was defined as the change in 0.001 absorbance units per minute at 290 nm. The specific activity was expressed as units per gram of fresh weight.

2.5.5. PPO activity

For the determination of PPO activity, skinless pulp tissues (2 g) prepared were homogenized with 8 mL of ice-cold 200 mmol L^{-1} citric acid buffer (pH 6.8) containing 1% (w/v) polyvinyl–polypyrrolidone (PVPP) and centrifuged at 10,000 rpm for 15 min at 4 °C. PPO activity was evaluated according to the method described by Tian et al. (2002). The assay was performed by using 2 mL of citric acid buffer (pH 6.8), 1 mL of 100 mmol L^{-1} 4-methylcatechol and 2 mL of the supernatant. The increase in absorbance at 398 nm at 25 °C within 2 min was recorded. The specific activity was expressed as units per gram of fresh weight.

2.5.6. β-1,3-Glucanase activity

β-1,3-Glucanase was assayed by measuring the amount of reducing sugar released from the substrate by the method reported by Abeles et al. (1971). For the determination, skinless pulp tissues (2 g) prepared were homogenized with 8 mL of ice-cold 50 mmol L^{-1} citric acid/disodium hydrogen phosphate buffer (pH 4.8) containing 1% (w/v) polyvinyl–polypyrrolidone (PVPP) and centrifuged at 10,000 rpm for 15 min at 4 °C. The assay was assayed by incubating 1 mL of the enzyme supernatant citric acid/disodium hydrogen phosphate buffer (pH 4.8), 1 mL of 0.4% laminarin (w/v) and 1 mL of citric acid/disodium hydrogen phosphate buffer (pH 4.8) for 60 min at 37 °C and 1.5 mL 3,5-dinitrosalicylic acid (DNS) was added, then put in boiling water for 5 min. One unit was defined as the change in 0.001 absorbance units per minute at 530 nm. The specific activity was expressed as units per gram of fresh weight.

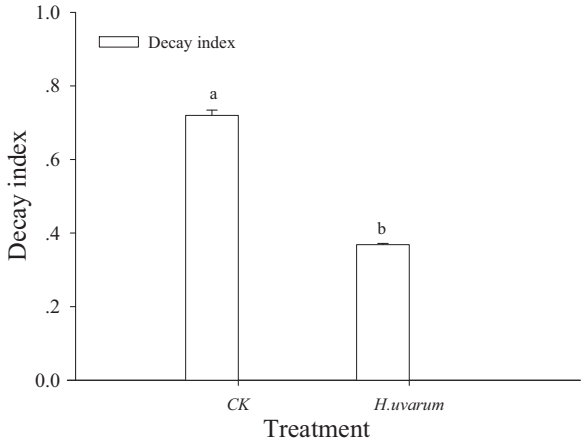

Fig. 1. The effect of pre-harvest application of antagonist yeast on the decay index of strawberries during storage at 2 °C and 95% RH for 15 days. Vertical bars represent standard errors of the means of three replications. The different letters (a and b) above the bars indicate significantly different scores, according to Duncan's multiple range tests at $P < 0.05$ level.

2.6. Analysis of data

All statistical analyses were performed in the SAS Software (Version 8.2; SAS Institute, Cary, NC, USA). The data were analyzed by one-way analysis of variance (ANOVA). Comparison of means was performed by Duncan's multiple range tests. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Efficacy of H. uvarum on decay index of strawberries

Treatment with preharvest application of *H. uvarum* at 1×10^8 CFU mL^{-1} could significantly reduce the incidence of mold decay in strawberries, compared with the control after storage at 2 ± 1 °C and 90–95% RH for 15 days (Fig. 1). The decay index showed a 2-fold reduction over the water control.

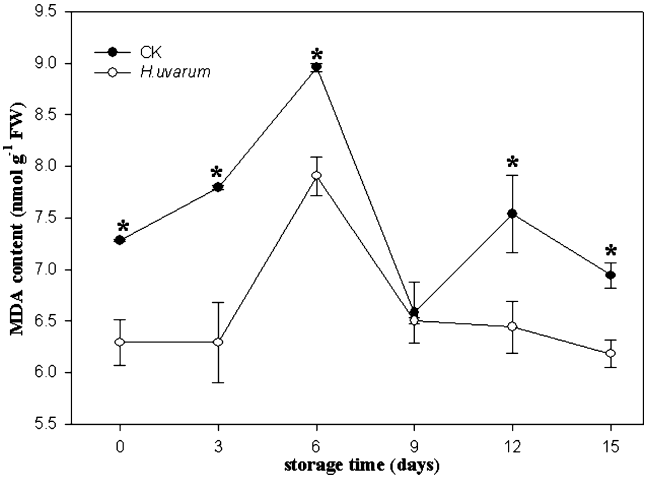

Fig. 2. The effect of pre-harvest application of antagonist yeast on the MDA content of strawberries during storage at 2 °C and 95% RH for 15 days. Vertical bars represent standard errors of the means of three replications. The "*" above the bars indicate significantly different scores, according to Duncan's multiple range tests at $P < 0.05$ level.

Table 1
The effect of pre-harvest application of antagonist yeast on the firmness, TSS and PH of strawberries during storage at 2 °C and 95% RH for 15 days.

Storage time (days)	F (*N*)		TSS (%)		pH	
	Control	*H. uvarum*	Control	*H. uvarum*	Control	*H. uvarum*
0	3.61 ± 0.01a	3.81 ± 0.03b	6.72 ± 1.75a	7.40 ± 1.07b	3.86 ± 0.06a	3.87 ± 0.02a
3	3.35 ± 0.04a	3.49 ± 0.06a	7.51 ± 0.76a	9.67 ± 0.70b	3.65 ± 0.06a	3.66 ± 0.04a
6	3.00 ± 0.04a	3.22 ± 0.02ab	11.44 ± 1.12a	11.08 ± 1.22a	3.96 ± 0.06ab	3.89 ± 0.06b
9	2.75 ± 0.03a	2.84 ± 0.03b	10.75 ± 0.91a	11.70 ± 1.08b	4.64 ± 0.00a	4.50 ± 0.17a
12	1.96 ± 0.04a	2.51 ± 0.01ab	6.10 ± 0.73a	6.90 ± 0.88b	4.76 ± 0.02a	4.91 ± 0.00a
15	1.57 ± 0.05a	1.82 ± 0.02b	5.10 ± 0.73a	5.90 ± 0.88b	4.94 ± 0.03a	4.99 ± 0.13a

All the results were expressed as means ± standard deviation of three replications. Values in a column followed by different letters are significantly different scores, according to Duncan's multiple range test at $P < 0.05$ level.

3.2. Efficacy of *H. uvarum* on strawberry quality

The effect of preharvest spray treatment of *H. uvarum* on strawberry fruit firmness, TSS, and pH is shown in Table 1. During the 15 days of storage at 2 ± 1 °C and 90–95% RH, the fruit firmness was reduced and the total soluble solute content was increased in the first days and then reduced. However, the firmness of strawberries treated by *H. uvarum* decreased more slowly than that of the control, the total soluble solute content peak came on the 9th day, which was late and significantly higher than for the control, and there was no significant difference in pH and surface color (L^* values and a^* values); L^* values decreased from 42.35 ± 1.30 to 30.14 ± 1.90 and a^* values from 41.21 ± 1.14 to 29.18 ± 1.91.

3.3. Efficacy of *H. uvarum* on MDA content

The MDA content gradually increased in the first 6 days during storage (Fig. 2), and then began to decrease. The MDA content of strawberries treated with *H. uvarum* was lower than that of the control at all the storage periods. Especially at the 3rd day of storage, the MDA content of strawberries treated with *H. uvarum* was reduced 19.3% of that in the control, but no significant difference was found at the 9th day.

3.4. Efficacy of *H. uvarum* on defense-related enzyme activities

The changes in SOD, POD, CAT and APX activities in response to *H. uvarum* are shown in Fig. 3 and the changes in PAL, PPO and

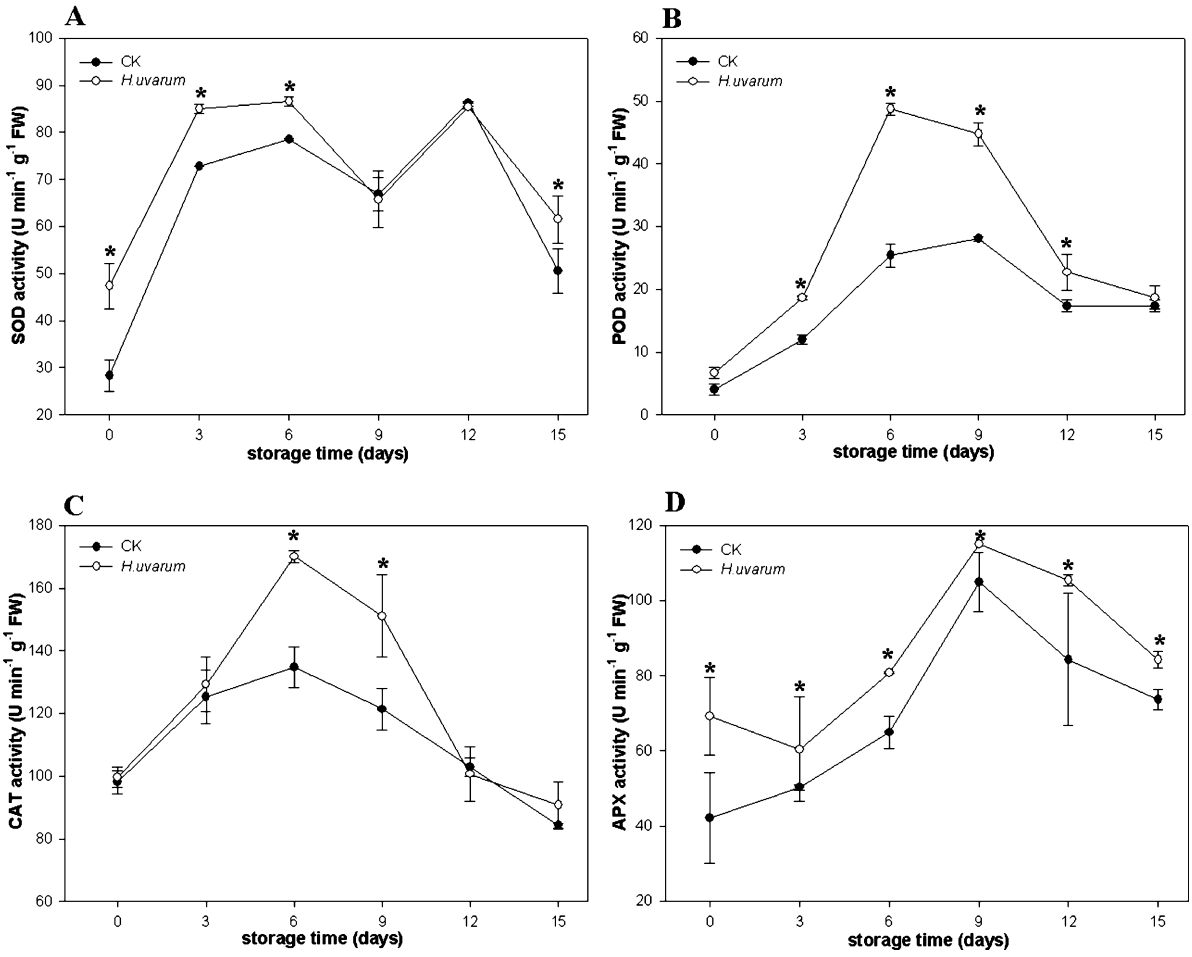

Fig. 3. The effect of pre-harvest application of antagonist yeast on the SOD, POD, CAT and APX activities of strawberries during storage at 2 °C and 95% RH for 15 days. Vertical bars represent standard errors of the means of three replications. The "*" above the bars indicate significantly different scores, according to Duncan's multiple range tests at $P < 0.05$ level.

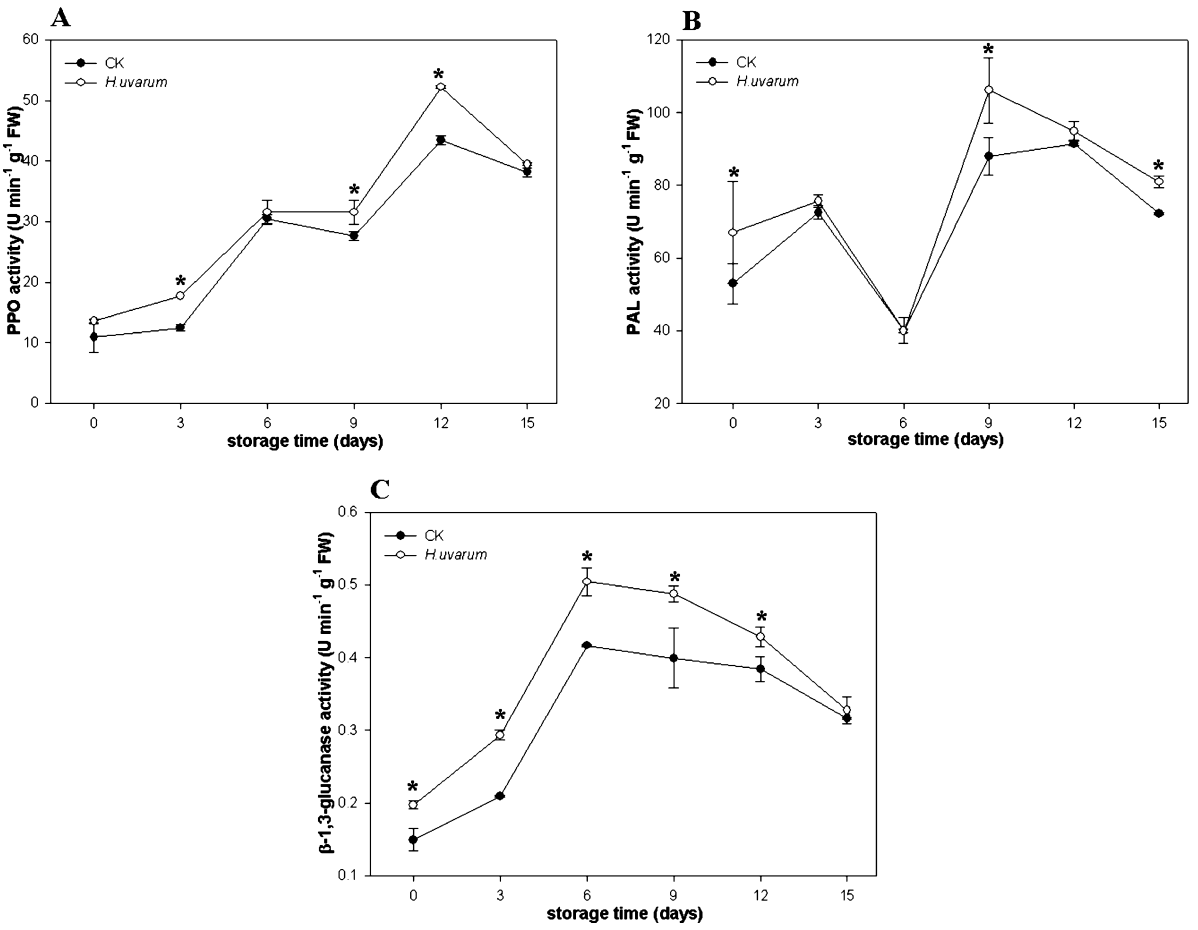

Fig. 4. The effect of pre-harvest application of antagonist yeast on the PAL, PPO and β-1,3-glucanase activities of strawberries during storage at 2 °C and 95% RH for 15 days. Vertical bars represent standard errors of the means of three replications. The "*" above the bars indicate significantly different scores, according to Duncan's multiple range tests at $P < 0.05$ level.

β-1,3-glucanase activities in Fig. 4. The SOD activities (Fig. 3A) under treatment with *H. uvarum* showed 1.6- and 1.2-fold increases over those in the water control at 0 and 3 days, respectively. Although SOD activity in yeast-treated tissues decreased to a low level at 9 days and was a little bit lower than that in the control, a significant increase in activity was observed at 12 days and 1.2-fold increase occurred at day 15 ($P < 0.05$). During the whole storage period, POD activity (Fig. 3B) under treatment with *H. uvarum* remained a stable higher level than the water control, especially on the 6th and 9th days, approximately 1.9- and 1.6-fold that of the water, respectively. In comparison to the control, the CAT (Fig. 3C) activity in the yeast-pretreated fruit was significantly higher at days 6 and 9 ($P < 0.05$), which was approximately 1.3- and 1.2-fold that of the water control, respectively. The APX (Fig. 3D) activity increased in strawberry fruit in the first 12 days of storage and was slightly reduced at the end of storage, and the yeast-treated tissues had more enzyme activity, about 1.4-fold of that of the control on the 3rd day.

In comparison to the control, the PAL activity (Fig. 4A) in the yeast-pretreated fruit was only higher at days 0 and 9, approximately 1.3- and 1.2-fold that of the water control, and there was no significant difference between the treatment and the control. On the day of harvest, the PPO (Fig. 4B) activities of treatmed fruit were significantly higher and almost 1.6-fold that of the water control ($P < 0.05$). During storage, the PPO activities were increasing over the first nine days, then lower, and the activities in treated fruit were basically 1.2-fold those of the water control. The trend of β-1,3-glucanase activities (Fig. 4C) was similar to that of PPO activities, only the peak was at the 6th day and the biggest difference betweeen the treatment and the control was found on the 3rd day, which was approximately 1.4-fold.

4. Discussion

Research on the effect of *H. uvarum* in controlling postharvest decay and its effect on quality parameters of fruit is scarce. Liu et al. (2010c) found that the antagonist *H. uvarum* used alone significantly inhibited spore germination and lesion diameters of *B. cinerea* in vitro, reduced natural decay development of grape berries, and did not impair quality parameters. In addition, NH_4-Mo (Liu et al., 2010d) and tea polyphenol (Liu et al., 2010b) could improve the efficacy of *H. uvarum*. However, this is the first time that preharvest application of *H. uvarum* has been used on strawberries and the results indicate that *H. uvarum* appears to significantly reduce fruit decay and induce disease resistance, which might be associated with the increased activities of SOD, CAT, POD, PPO, PAL, APX and β-1,3-glucanase in the fruit.

Plants under stress will induce accumulation of reactive oxygen species (ROS) and stimulate disease resistance reaction (Thoma et al., 2003). The accumulation of ROS has the potential to serve as resistance against invading pathogens and as signals for activating further plant defense reactions (Lamb and Dixon, 1997), although ROS overproduction can lead to lipid peroxidation and cause

oxidative damage (Thoma et al., 2003). It is well known that plants can induce effective antioxidant systems to protect themselves against oxidation damage. SOD, CAT, POD and APX are associated with ROS metabolism (Zhao et al., 2008; Liu et al., 2010a; Maya and Matsubara, 2013) and MDA is the end product of lipid peroxidation (Ge et al., 2010). A higher level of SOD, CAT, POD and APX activities and a lower level of MDA content are equal to a more effective antioxidant defense system and closely associated with delayed senescence in harvested fruit. Our results showed that SOD, CAT, POD and APX activities of strawberries treated with *H. uvarum* were higher than those of the control, and MDA content of treated strawberries was lower than that of the control at most storage periods. Especially on the 6th day during storage, the CAT and POD activity in the yeast-pretreated fruit were 1.3- fold and 1.9-fold that of the water control, respectively. This indicates that preharvest application of *H. uvarum* had a significant effect on increasing the antioxidative activity of the fruit.

The metabolism of the phenylpropanoid pathway is known to be influenced significantly by biocontrol agents and postharvest pathogens (Ballester et al., 2012, 2011; Hershkovitz et al., 2012). The key enzyme PAL in this pathway catalyzes the conversion of phenylalanine to trans-cinnamic acid, a key intermediate in the synthesis of salicylic acid. Moreover, PAL plays important roles in biosynthesis of phenolics, phytoalexins and lignin, the key factors responsible for disease resistance (Joe et al., 2012). PPO is a copper containing enzyme, which oxidizes phenolics to highly toxic quinines and is involved in the terminal oxidation of diseased plant tissue, and which is attributed for its role in disease resistance. In this experiment, antagonistic yeast treatment could enhance PPO and PAL activities during storage. Consequently, we suggest that preharvest application of *H. uvarum* could be associated with promoting high values of lignin contents, which increases the resistance of plant cell walls against pathogens, and on the other hand, limits pathogenic bacteria access to water and nutrients from the host.

The synthesis of pathogenesis-related proteins is thought to be another important mechanism of resistance to various diseases. β-1,3-glucanase is the most fully characterized PR protein, which acts directly by degrading the cell walls of the pathogen or indirectly by releasing oligosaccharides, elicitors of defense reactions, both of which are potential defense mechanisms against fungal infection (Liu et al., 2010a). Our results showed that the activity of β-1,3-glucanase was significantly stimulated by the treatment, which mean pre-harvest spraying the yeast could enable strawberries to accumulate sufficient β-1,3-glucanase to help stimulate the defense system in senescing fruit.

In addition to disease control, the main quality parameters including firmness, TSS, pH and surface color of strawberry fruit were also assayed. It was found that preharvest *H. uvarum* treatments maintained fruit firmness, ascorbic acid and TSS, and did not impair pH and surface color during postharvest storage. These results indicated that *H. uvarum* might have potential for control of major postharvest decay and prolong the shelf-life of strawberries, although more experiments need to be carried out. Further research should focus on studying the detailed mechanisms of action of *H. uvarum* using DNA microarrays and high-throughput sequencing technologies and identifying approaches to enhance efficacy.

5. Conclusions

Results from the present study showed that the preharvest spraying of the antagonist *H. uvarum* did not significantly induce effects on surface color parameters and pH of strawberries, but significantly reduced microbiological decay incidence, maintained the firmness and TSS of strawberries, and improved the quality and storage properties of the fruit throughout storage. The mechanism by which this antagonist inhibited postharvest decay might be closely related to its ability to increase the activities of defense-related enzymes. However, the detailed modes of its mechanisms need to be elucidated further. In addition, with regard to application in practice, the biocontrol efficacy of the preharvest application of *H. uvarum* requires to be further enhanced.

Acknowledgments

This study was supported by Science and Technology Department of Jiangsu Province (No BE2010385) and A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions and National College Students' innovation and entrepreneurship (No. 201310307046).

References

Abeles, F., Bosshart, R., Forrence, L., Habig, W., 1971. Preparation and purification of glucanase and chitinase from bean leaves. Plant Physiol. 47, 129–134.
Assis, J.S., Maldonado, R., Muñoz, T., Escribano, M., Merodio, A.I.C., 2001. Effect of high carbon dioxide concentration on PAL activity and phenolic contents in ripening cherimoya fruit. Postharvest Biol. Technol. 23, 33–39.
Ballester, A.R., Lafuente, T.M., de Vos, R.C., Bovy, A.G., González-Candelas, L., 2012. Citrus phenylpropanoids and defense against pathogens. Part I: Metabolic profiling in elicited fruits. Food Chem. 136, 178–185.
Ballester, A.R., Lafuente, M.T., Forment, J., Gadea, J., De Vos, R.C., Bovy, A.G., González-Candelas, L., 2011. Transcriptomic profiling of citrus fruit peel tissues reveals fundamental effects of phenylpropanoids and ethylene on induced resistance. Mol. Plant Pathol. 12, 879–897.
Chan, Z., Tian, S., 2005. Interaction of antagonistic yeasts against postharvest pathogens of apple fruit and possible mode of action. Postharvest Biol. Technol. 36, 215–223.
Du, Z., Bramlage, W.J., 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric. Food Chem. 40, 1566–1570.
El-Neshawy, S.M., Shetaia, Y.M.H., 2003. Biocontrol capability of *Candida* spp. against Botrytis rot of strawberries with respect to fruit quality. In: Tijskens, L.M.M., Vollebregt, H.M. (Eds.), Proceedings of the International Conference on Quality in Chains. Vols. 1 and 2: An Integrated View on Fruit and Vegetable Quality. , pp. 727–733.
Fan, Y., Xu, Y., Wang, D., Zhang, L., Sun, J., Sun, L., Zhang, B., 2009a. Effect of alginate coating combined with yeast antagonist on strawberry (*Fragaria* × *ananassa*) preservation quality. Postharvest Biol. Technol. 53, 84–90.
Fan, Y., Xu, Y., Wang, D., Zhang, L., Sun, J., Sun, L., Zhang, B., 2009b. Effect of alginate coating combined with yeast antagonist on strawberry (*Fragaria* × *ananassa*) preservation quality. Postharvest Biol. Technol. 53, 84–90.
Fiori, S., Scherm, B., Liu, J., Farrell, R., Mannazzu, I., Budroni, M., Maserti, B.E., Wisniewski, M.E., Migheli, Q., 2012. Identification of differentially expressed genes associated with changes in the morphology of *Pichia fermentans* on apple and peach fruit. FEMS Yeast Res. 12, 785–795.
Ge, L., Zhang, H., Chen, K., Ma, L., Xu, Z., 2010. Effect of chitin on the antagonistic activity of *Rhodotorula glutinis* against *Botrytis cinerea* in strawberries and the possible mechanisms involved. Food Chem. 120, 490–495.
Hernández-Muñoz, P., Almenar, E., Ocio, M.J., Gavara, R., 2006. Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria* × *ananassa*). Postharvest Biol. Technol. 39, 247–253.
Hershkovitz, V., Ben-Dayan, C., Raphael, G., Pasmanik-Chor, M., Liu, J., Belausov, E., Aly, R., Wisniewski, M., Droby, S., 2012. Global changes in gene expression of grapefruit peel tissue in response to the yeast biocontrol agent *Metschnikowia fructicola*. Mol. Plant Pathol. 13, 338–349.
Joe, M.M., Islam, M.R., Karthikeyan, B., Bradeepa, K., Sivakumaar, P.K., Sa, T., 2012. Resistance responses of rice to rice blast fungus after seed treatment with the endophytic *Achromobacter xylosoxidans* AUM54 strains. Crop Prot. 42, 141–148.
Karabulut, O.A., Tezcan, H., Daus, A., Cohen, L., Wiess, B., Droby, S., 2004. Control of preharvest and postharvest fruit rot in strawberry by *Metschnikowia fructicola*. Biocontrol Sci. Technol. 14, 513–521.
Kochba, J., Lavee, S., Spiegel-Roy, P., 1977. Differences in peroxidase activity and isoenzymes in embryogenic ane non-embryogenic 'Shamouti' orange ovular callus lines. Plant Cell Physiol. 18, 463–467.
Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. Ann. Rev. Plant Biol. 48, 251–275.
Liu, F., Tu, K., Shao, X., Zhao, Y., Tu, S., Su, J., Hou, Y., Zou, X., 2010a. Effect of hot air treatment in combination with *Pichia guilliermondii* on postharvest anthracnose rot of loquat fruit. Postharvest Biol. Technol. 58, 65–71.
Liu, H.M., Guo, J.H., Cheng, Y.J., Liu, P., Long, C.A., Deng, B.X., 2010b. Inhibitory activity of tea polyphenol and *Hanseniaspora uvarum* against *Botrytis cinerea* infections. Lett. Appl. Microbiol. 51, 258–263.
Liu, H.M., Guo, J.H., Cheng, Y.J., Luo, L., Liu, P., Wang, B.Q., Deng, B.X., Long, C.A.A., 2010c. Control of gray mold of grape by *Hanseniaspora uvarum* and its effects on postharvest quality parameters. Ann. Microbiol. 60, 31–35.

Liu, H.M., Guo, J.H., Luo, L., Liu, P., Wang, B.Q., Cheng, Y.J., Deng, B.X., Long, C.A., 2010d. Improvement of *Hanseniaspora uvarum* biocontrol activity against gray mold by the addition of ammonium molybdate and the possible mechanisms involved. Crop Prot. 29, 277–282.

Liu, J., Sui, Y., Wisniewski, M., Droby, S., Liu, Y., 2013. Review: utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. Int. J. Food Microbiol. 167, 153–160.

Liu, J., Wisniewski, M., Droby, S., Tian, S., Hershkovitz, V., Tworkoski, T., 2011a. Effect of heat shock treatment on stress tolerance and biocontrol efficacy of *Metschnikowia fructicola*. FEMS Microbiol. Ecol. 76, 145–155.

Long, C.-A., Yuan, G., 2009. *Kloeckera apiculata* strain (34-9) to control *Botrytis cinerea* during the pre- and postharvest handling of strawberries. Ann. Microbiol. 59, 77–81.

Luna, C.M., González, C.A., Trippi, V.S., 1994. Oxidative damage caused by an excess of copper in oat leaves. Plant Cell Physiol. 35, 11–15.

Macarisin, D., Droby, S., Bauchan, G., Wisniewski, M., 2010. Superoxide anion and hydrogen peroxide in the yeast antagonist–fruit interaction: a new role for reactive oxygen species in postharvest biocontrol? Postharvest Biol. Technol. 58, 194–202.

Maya, M.A., Matsubara, Y.-I., 2013. Tolerance to *Fusarium wilt* and anthracnose diseases and changes of antioxidative activity in mycorrhizal cyclamen. Crop Prot. 47, 41–48.

McGuire, R.G., 2000. Population dynamics of postharvest decay antagonists growing epiphytically and within wounds on grapefruit. Phytopathology 90, 1217–1223.

Mekbib, S.B., Regnier, T.J., Korsten, L., 2011. Efficacy and mode of action of yeast antagonists for control of *Penicillium digitatum* in oranges. Trop. Plant Pathol. 36, 233–240.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.

Nunes, C.A., 2012. Biological control of postharvest diseases of fruit. Eur. J. Plant Pathol. 133, 181–196.

Palou, L., Usall, J., Smilanick, J.L., Aguilar, M.J., Vinas, I., 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Manag. Sci. 58, 459–466.

Romanazzi, G., Nigro, F., Ippolito, A., Salerno, M., 2001. Effect of short hypobaric treatments on postharvest rots of sweet cherries, strawberries and table grapes. Postharvest Biol. Technol. 22, 1–6.

Saravanakumar, D., Ciavorella, A., Spadaro, D., Garibaldi, A., Gullino, M.L., 2008. *Metschnikowia pulcherrima* strain MACH1 outcompetes *Botrytis cinerea, Alternaria alternata* and *Penicillium expansum* in apples through iron depletion. Postharvest Biol. Technol. 49, 121–128.

Thoma, I., Loeffler, C., Sinha, A.K., Gupta, M., Krischke, M., Steffan, B., Roitsch, T., Mueller, M.J., 2003. Cyclopentenone isoprostanes induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation in plants. Plant J. 34, 363–375.

Tian, S., Xu, Y., Jiang, A., Gong, Q., 2002. Physiological and quality responses of longan fruit to high O_2 or high CO_2 atmospheres in storage. Postharvest Biol. Technol. 24, 335–340.

Wang, Y.S., Tian, S.P., Xu, Y., Qin, G.Z., Yao, H., 2004. Changes in the activities of pro- and anti-oxidant enzymes in peach fruit inoculated with *Cryptococcus laurentii* or *Penicillium expansum* at 0 or 20 °C. Postharvest Biol. Technol. 34, 21–28.

Xu, B., Zhang, H., Chen, K., Xu, Q., Yao, Y., Gao, H., 2013. Biocontrol of postharvest Rhizopus decay of peaches with *Pichia caribbica*. Curr. Microbiol., 1–7.

Xu, X., Qin, G., Tian, S., 2008. Effect of microbial biocontrol agents on alleviating oxidative damage of peach fruit subjected to fungal pathogen. Int. J. Food Microbiol. 126, 153–158.

Yu, T., Yu, C., Lu, H., Zunun, M., Chen, F., Zhou, T., Sheng, K., Zheng, X., 2012. Effect of *Cryptococcus laurentii* and calcium chloride on control of *Penicillium expansum* and *Botrytis cinerea* infections in pear fruit. Biol. Control 61, 169–175.

Zhang, H., Wang, L., Dong, Y., Jiang, S., Cao, H., Meng, R., 2007a. Postharvest biological control of gray mold decay of strawberry with *Rhodotorula glutinis*. Biol. Control 40, 287–292.

Zhang, H., Zheng, X., Wang, L., Li, S., Liu, R., 2007b. Effect of yeast antagonist in combination with hot water dips on postharvest Rhizopus rot of strawberries. J. Food Eng. 78, 281–287.

Zhao, Y., Tu, K., Shao, X., Jing, W., Su, Z., 2008. Effects of the yeast *Pichia guilliermondii* against *Rhizopus nigricans* on tomato fruit. Postharvest Biol. Technol. 49, 113–120.

Zheng, X., Chen, J., 2009. Microarray analysis of gene expression profile induced by the biocontrol yeast *Cryptococcus laurentii* in cherry tomato fruit. Gene 430, 12–16.

Zong, Y., Liu, J., Li, B., Qin, G., Tian, S., 2010. Effects of yeast antagonists in combination with hot water treatment on postharvest diseases of tomato fruit. Biol. Control 54, 316–321.